Carcinogenic Effect of Dinitrosopiperazine in Adult Swiss Mice and after Transplacental or Translactational Exposure

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ABSTRACT

Dinitrosopiperazine, which can be formed from several commercial products, including Triforin (N,N'-piperazinidylbis-2,2,2-trichloroethylidene bisformamide), was found to be carcinogenic in adult Swiss mice, and in their transplacentally and translactationally exposed offspring. Adults were treated for 1 year, pregnant mothers in the third trimester of pregnancy, and lactating mothers for the first 20 days postpartum. Deaths attributable to the acute toxic effect of the carcinogen treatment amounted to 15% among the treated adult animals and to 57% in the transmaternally exposed animals. The incidence of tumors was significantly higher in the transmaternally exposed animals, and the latency time for appearance of tumors in treated adult animals was significantly shorter than in the controls. In untreated mice, the frequency of spontaneous tumors of all types was higher in females, whereas hepatocellular carcinomas in all treated groups and lung adenomas in treated adult animals and to 57% in the transmaternally exposed animals. The incidence of tumors was significantly higher in the transmaternally exposed animals, and the latency time for appearance of tumors in treated adult animals was significantly shorter than in the controls. In untreated mice, the frequency of spontaneous tumors of all types was higher in females, whereas hepatocellular carcinomas in all treated groups and lung adenomas in adult and in the translactationally exposed group appeared at a higher frequency in males. These observations show that exposure of pregnant or lactating mothers to carcinogens may result in an increased incidence of tumors in their offspring when they reach maturity.

INTRODUCTION

In the presence of inorganic nitrite, secondary and some tertiary amines and amides undergo N-nitrosation reactions under certain conditions to produce N-nitroso derivatives, which are often found to be carcinogenic in animal systems. These compounds represent a possible environmental carcinogenic hazard for humans as well. It has been demonstrated that, if pregnant animals are exposed to carcinogens, the fetus will also be affected as a result of the transplacental passage of the toxic agent. Thus, the offspring often develop tumors (1, 4, 7, 9, 11, 14). Also, chemical carcinogens can enter the mother’s milk, and as a result of this exposure the F1 generation has been shown to exhibit increased tumor incidence (3, 10, 11).

In the present study, the tumorigenic effect of D-NPZ3 on adult Swiss mice and on their progeny was examined.

MATERIALS AND METHODS

The Carcinogen. D-NPZ was prepared from the fungicide Triforin (N,N'-piperazinidylbis-2,2,2-trichloroethylidene bisformamide; Sapor, Funginex, Celamerck, Ingelheim, Federal Republic of Germany), according to our previously published procedure (2). D-NPZ was administered to animals in drinking water.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>Av. life span</th>
<th>Tumor-bearing animals</th>
<th>Av. survival time for animals with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Days</td>
<td>p (t test)</td>
</tr>
<tr>
<td>AF, (untreated controls)</td>
<td>80</td>
<td>79</td>
<td>397</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male</td>
<td>51</td>
<td>50</td>
<td>294</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>29</td>
<td>254</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B (carcinogen-treated</td>
<td>50</td>
<td>36</td>
<td>254</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>adults)</td>
<td>Male</td>
<td>25</td>
<td>22</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>14</td>
<td>361</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CF, (transplacentally</td>
<td>26</td>
<td>25</td>
<td>361</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>exposed)</td>
<td>Male</td>
<td>12</td>
<td>12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>13</td>
<td>28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DF, (translactationally</td>
<td>30</td>
<td>30</td>
<td>356</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>exposed)</td>
<td>Male</td>
<td>19</td>
<td>19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Both sexes combined.

For statistical significance tests: AF, versus B, CF, or DF, (both sexes combined).

1 This investigation was carried out under a collaborative research agreement with the International Agency for Research on Cancer (RA/75/014).
2 To whom requests for reprints should be addressed.

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Results of the experiments are summarized in Tables 1

and 2. The following additional details serve to clarify the data. The average number of siblings per mother in the AF group was 9.1 at birth, which decreased to 8.0 at the time of separation; whereas, for the CF group, the respective numbers were 5.8 and 2.6. In the DF group, the average number of siblings per mother was 9.3 at birth, which decreased to 3.0 by the 21st day postpartum.

Thus, among the transmaternally treated animals (combined CF, and DF, groups), 68% of the animals died at the neonatal stage. In Group B, treated with D-NPZ, 28% of animals died before the appearance of the first tumor.

The most common early spontaneous tumor type in Group AF was lymphoid leukemia (33%), while lung adenomas (13%) and hepatocellular carcinomas (8%) were found only in older animals.

The average life span in Group B was significantly shorter than that of the control group, as well as those of the CF, and DF groups. The average survival time for animals with tumors in general was significantly shorter in the treated Group B; in particular, the frequency of lung adenomas was significantly increased (39%).

A significantly high percentage of tumors in Group CF were lymphomas and leukemias, characterized by splenomegaly and enlarged thymus and lymph nodes.

In Group DF, the incidence of tumors was significantly increased in the sucklings, and their survival time was significantly shortened. Histological examination of liver tumors revealed well-differentiated and fairly well-differentiated hepatocellular carcinomas.

It is noteworthy to consider the differing susceptibilities of the sexes to the carcinogen. Whereas the spontaneous tumor incidence was 2.3 times higher for the females than for males in the control group, the total tumor incidence in Groups B and CF was nearly the same for both sexes. In comparison, the males in Group DF developed a higher incidence of tumors. In general, leukemias occurred preferentially in the females in all groups. Hepatocellular carcinoma incidence was especially prevalent in males of Group DF, whereas none of the females in Groups B and CF were affected. The overall tumor frequency among males in Group CF, and DF, was 2.5 times higher than among the males of the control group.

Statistical evaluation of the tumorigenicity data on the carcinogen-treated pregnant mice was not possible, because of the small number of animals. Tumors in Groups B, CF0, and DF0 were histologically identical to those induced in offspring exposed transplacentally or transductantly.

Discussion

On the basis of the evidence to date, namely, that Triforin can be nitrosatively converted to D-NPZ, that Triforin in the presence of nitrite is tumorigenic to mice (2), and that D-NPZ is tumorigenic to mice through transmaternal exposure, it is recommended that administration of piperazine-containing drugs and compounds be minimized or eliminated during pregnancy.

These tumorigenic properties of D-NPZ are well recognized in several animals species (13, 14).

In a fashion similar to that of other carcinogens (8), D-NPZ...
or its active metabolite can enter the circulatory system and pass through the placenta, exerting embryotoxic or teratogenic effects and inducing tumors in the offspring (1, 3, 4, 7, 9, 11, 14). In our experiments, animals in Group CF, showed an increased incidence of lung adenomas and lymphomas. Our recent studies also demonstrate that sucklings of postnatally treated lactating mice (Group DF) exhibited an increased frequency of lung adenomas (63%) and hepatocellular carcinomas (53%).

Enzymes, at least certain ones, are required for carcinogenicity; however, maternal metabolism has not been evaluated for its potential contribution to fetal risk. D-NPZ is most probably not a directly acting carcinogen and requires metabolic activation, probably by the mother. A partial explanation for the high tumor frequency in the prenatally and neonatally exposed group could be that the metabolizing enzyme levels are low in the fetal state and in the neonatal state at 4 weeks postpartum (5). Therefore, the same amount of carcinogen may create a higher risk of exposure to the fetus and to neonates than to adult animals (5, 12). Increased incidence of hepatomas in male Swiss mice after transplacental exposure to indirectly acting carcinogens has been reported previously (12). An additional factor that may influence the frequency of tumors in the pre- and neonatally exposed animals is that the development and induction of a number of liver enzymes are androgen dependent (5, 6). The ultimate expression of carcinogenic potency is also modulated by the DNA-repairing ability of the target tissues at various stages of their development (12).

This is the first report of perinatal carcinogenesis by a heterocyclic nitrosamine, although perinatal carcinogenesis by nitrosamines is well established (11).

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REFERENCES

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